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D_1 Dopamine Receptor Agonists Mediate Activation of p38 Mitogen-Activated Protein Kinase and c-Jun Amino-Terminal Kinase by a Protein Kinase A-Dependent Mechanism in SK-N-MC Human Neuroblastoma Cells

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ABSTRACT

We investigated the effects of D_1 dopamine receptor stimulation on the activation of mitogen-activated protein kinases (MAPKs) in SK-N-MC human neuroblastoma cells. We found that the D_1 dopamine receptor agonist SKF38393 induced similar time- and dose-related activation of p38 MAPK and c-Jun amino-terminal kinase (JNK), whereas extracellular signal-regulated kinase activity was not affected by D_1 dopamine receptor stimulation. Maximal stimulation of p38 MAPK and JNK was observed after a 15-min incubation with 100 μM SKF38393. In contrast, 10 μM quinpirole, a D_2 dopamine receptor agonist, did not activate p38 MAPK or JNK. Treatment of cells with 10 μM SCH23390, a D_1 dopamine receptor antagonist, significantly inhibited the activation of both kinases by SKF38393. These results indicate that activation of the p38 MAPK and JNK signaling pathways is mediated by dopamine D_1 receptors in SK-N-MC neuroblastoma cells. Furthermore, dibutyryl-cAMP mimicked SKF38393-mediated stimulation of p38 MAPK and JNK. Inhibition of protein kinase A by 1 μM H-89 or 10 μM adenosine 3′,5′-cyclic monophosphothioate (Rp-isomer, triethylammonium salt) markedly attenuated the activation of p38 MAPK and JNK. Conversely, the selective protein kinase C inhibitor calphostin C did not block D_1 dopamine receptor-stimulated activation of p38 MAPK and JNK. These results demonstrate, for the first time, that the G_s-coupled D_1 dopamine receptor activates the p38 MAPK and JNK signaling pathways by a protein kinase A-dependent mechanism.

MAPKs have been implicated in the transduction of a wide variety of extracellular signals. In mammalian cells, at least three subgroups of MAPKs have been identified (i.e., ERK, JNK, and p38 MAPK). These kinases, which are activated by distinct extracellular stimuli through independent signaling pathways, serve different functions (Johnson and Vaillancourt, 1994; Cobbs and Goldsmith, 1995; Treisman, 1996; Paul *et al.*, 1997). ERK can be activated by receptor tyrosine kinases (e.g., growth factor receptors) and by stimulation of GPCRs (Cleasson-Welsh, 1994; Koch *et al.*, 1994; Blesen *et al.*, 1995; Cobbs and Goldsmith, 1995). JNK and p38 MAPK are activated by stimuli such as UV irradiation, osmotic stress, and inflammatory cytokines (Paul *et al.*, 1997). Recent studies indicate that G proteins are also involved in the regulation of p38 MAPK and JNK pathways. For instance, $G_{\alpha 12}$ and $G_{\alpha 13}$ have been shown to activate JNK in COS-7 cells, as well as in p19 embryonic carcinoma cells (Jho *et al.*, 1997; Voyno-Yasenetskaya *et al.*, 1997). Activation of G_q/G_{11}, G_i, and G_s was reported to mediate p38 MAPK stimulation (Yamauchi *et al.*, 1997). However, the signaling cascades for the activation of p38 MAPK and JNK have not been completely characterized. It has been established that ERK plays an essential role in regulating cell proliferation and differentiation (Johnson and Vaillancourt, 1994; Cobbs and Goldsmith, 1995; Treisman, 1996). In contrast, p38 MAPK and

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ABBREVIATIONS: MAPK, mitogen-activated protein kinase; ERK, extracellular signal-regulated kinase; JNK, c-Jun amino-terminal kinase(s); PKA, protein kinase A; MBP, myelin basic protein; PKC, protein kinase C; GPCR, G protein-coupled receptor; DHX, dihydrexidine; MKK, mitogen-activated protein kinase kinase; GST, glutathione *S*-transferase; EGTA, ethylene glycol bis(β-aminoethyl ether)-*N*,*N*,*N*′,*N*′-tetraacetic acid; Rp-cAMPS, adenosine 3′,5′-cyclic monophosphothioate, Rp-isomer, triethylammonium salt.

JNK have been shown to mediate cell death induced by deprivation of nerve growth factor in PC-12 cells (Xia *et al.*, 1995), by ceramide in U937 cells (Verheij *et al.*, 1996), by anti-IgM antibody in human B lymphocytes (Graves *et al.*, 1996), or by glutamate in rat cerebellar granular cells (Kawasaki *et al.*, 1997). Furthermore, these kinases were shown to play a role in cell cycle regulation and in the biosynthesis of nitric oxide (Rao and Runge, 1996; Molnar *et al.*, 1997; Silva *et al.*, 1997).

Dopamine receptors constitute a subfamily of GPCRs. At least five dopamine receptor subtypes [D_1-like (i.e., D_1 and D_5) and D_2-like (i.e., D_2, D_3, and D_4)] have been identified. It has been demonstrated that D_1-like receptors exert their actions by stimulating cellular adenylyl cyclase via G_s, by affecting ion channels, or by modulating phospholipase C activity (Rogue and Malviya, 1994; Wang *et al.*, 1995; Yu *et al.*, 1996). In contrast, activation of D_2-like dopamine receptors results in inhibition of adenylyl cyclase and activation of K^+ channels (Israel *et al.*, 1985). Recent evidence indicates that tyrosine phosphorylation may also play an important role in mediating signals initiated by dopamine receptors. For instance, the mitogenic response induced by D_2 dopamine receptor activation in Chinese hamster ovary cells was associated with enhanced tyrosine phosphorylation (Lajiness *et al.*, 1993). Furthermore, ERK activation was demonstrated in a cellular system in which D_2 dopamine receptors were overexpressed (Yan *et al.*, 1997), whereas D_1 receptor stimulation was shown to inhibit MAPK activation elicited by platelet-derived growth factor in vascular smooth muscle cells (Yasunari *et al.*, 1997). These findings indicate that MAPK participates in dopamine receptor signaling. However, the role of p38 MAPK and JNK in D_1 dopamine receptor signaling has not been previously described.

In the present study, we investigated whether the MAPKs ERK, p38 MAPK, and JNK are involved in D_1 dopamine receptor signaling cascades in SK-N-MC human neuroblastoma cells, which express high densities of D_1 dopamine receptors. We report here that the D_1 dopamine receptor agonists SKF38393 and DHX activate p38 MAPK and JNK, but not ERK, in a dose- and time-dependent fashion. Furthermore, this activation is mediated via a PKA-dependent pathway.

Experimental Procedures

Materials. SKF38393 was obtained from RBI (Natick, MA), DHX hydrochloride was from Tocris Cookson (Baldwin, MO), and dibutyryl-cAMP, H-89, and Rp-cAMPs were purchased from Calbiochem (La Jolla, CA). MBP and genistein were purchased from Sigma Chemical Co. (St. Louis, MO). Electrophoresis reagents were obtained from Bio-Rad (Richmond, CA). Agarose-conjugated antiphosphotyrosine antibody (clone 4G10), anti-human phospho-c-Jun (Ser73), anti-rat MAP R2, and anti-human c-Jun(1–169)-GST were obtained from Upstate Biotechnology (Lake Placid, NY). Anti-p38, anti-ERK2, and protein A/G were purchased from Santa Cruz Biotech (Santa Cruz, CA). Horseradish peroxidase-linked, anti-rabbit IgG, secondary antibodies were obtained from Pierce (Rockford, IL). Other chemicals were purchased from standard laboratory suppliers and were of the highest purity available.

Cell culture and preparation of cell lysates. SK-N-MC human neuroblastoma cells were obtained from the American Type Culture Collection (Rockville, MD). The cells were maintained in Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% sodium pyruvate, in a humidified atmosphere of 95% room air/5% CO_2 at 37°. The day before experiments, the medium of cells that were 80–90% confluent was replaced with medium containing 0.5% fetal bovine serum. After treatment with various agents, cells were washed twice with cold phosphate-buffered saline and were lysed in lysis buffer (buffer A, containing 50 mM Tris·HCl, pH 7.4, 150 mM NaCl, 20 mM β-glycerophosphate, 1 mM EGTA, 20 mM NaF,

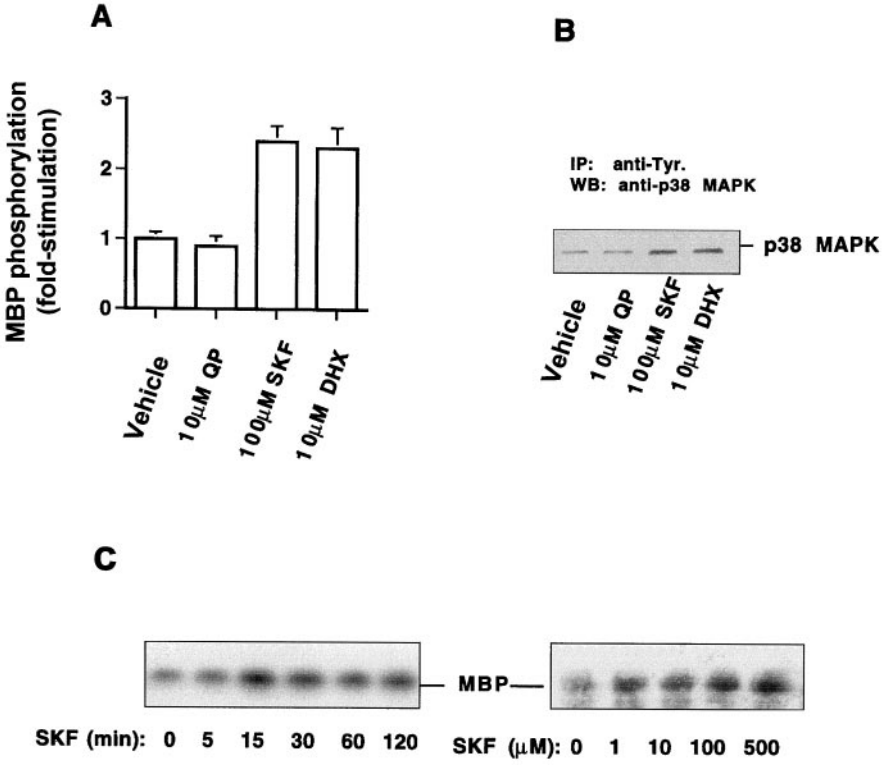

Fig. 1. Activation of p38 MAPK in SK-N-MC human neuroblastoma cells by D_1 dopamine receptor agonists. A, Cells were treated for 10 min with 10 μM quinpirole (*QP*), 100 μM SKF38393 (*SKF*), 10 μM DHX, or vehicle. Equal aliquots of cell lysates were immunoprecipitated with anti-p38 MAPK polyclonal antibody. Kinase activity was assayed by incubating lysates with [γ-^{32}P]ATP and MBP, and radioactivity incorporated into MBP was assessed by liquid scintillation counting. Results are expressed as mean ± standard error of three independent experiments. B, Cells were treated for 10 min with 10 μM quinpirole, 100 μM SKF38393, 10 μM DHX, or vehicle. Cell proteins (300 μg) were immunoprecipitated with antiphosphotyrosine (*anti-Tyr.*) antibody (4G10), precipitates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes, and phosphotyrosine protein-containing blots were then probed with anti-p38 MAPK antibody. *IP*, immunoprecipitation; *WB*, Western blotting. C, Cells were treated with 100 μM SKF38393 for the indicated times (*left*) or with various doses of SKF38393 for 15 min (*right*), and p38 MAPK activity was assayed. Autoradiograms are representative of three experiments each.

3 mM Na_3VO_4, 1 mM phenylmethylsulfonyl fluoride, 10 μg/ml aprotinin, 10 μg/ml leupeptin, and 1% Nonidet P-40). Lysates were centrifuged at 12,000 × *g* for 15 min at 4° to precipitate debris. The supernatant protein content was determined, and aliquots were used for immunoprecipitation or immunoblotting (see below).

***In vitro* kinase assays.** For immunoprecipitation, 1 μg of anti-p38 MAPK or 2 μg of anti-JNK antibodies were added to aliquots (200 μg of protein) of cell lysates and incubated overnight at 4°. After the addition of 15 μl of protein A/G, the tubes were incubated for an additional 2 hr. The precipitates were washed three times with buffer A and twice with buffer B (20 mM Tris, pH 7.5, 2 mM EGTA, 20 mM $MgCl_2$, 12.5 mM β-glycerophosphate, 1 mM dithiothreitol, 0.2 mM Na_3VO_4). Kinase activity was assessed in buffer B, in the presence of 50 μM [γ-^{32}P]ATP (5 μCi) and 2 μg of c-Jun(1–169)-GST or 0.3 mg/ml MBP, for JNK or p38 MAPK, respectively. After being shaken for 30 min at 30°, the reactions were terminated by the addition of Laemmli sample buffer. The products were resolved by 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. The gels were stained with Coomasie Blue, and the phosphorylated MBP or c-Jun products were detected by autoradiography. Alternatively, the radioactivity incorporated into MBP was determined by liquid scintillation counting, as previously described (13, 14). PKC activity was assayed exactly as previously described (24).

Analysis of phosphorylated ERK and p38. Tyrosine-phosphorylated ERK and p38 were determined by incubation of 300 μg of supernatant protein overnight at 4° with 10 μl of agarose-conjugated, antiphosphotyrosine monoclonal antibody 4G10. The immunoprecipitates were collected, washed three times with buffer A, resuspended in 40 μl of sample buffer, and boiled for 5 min, and the proteins were separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. Phosphotyrosine-containing proteins were transferred to nitrocellulose membranes and immunoblotted using anti-ERK2 antibody. The signals were detected with the Supersignal Western blot detection system (Pierce).

Immunohistochemical localization of phosphorylated c-Jun. Immunostaining for phospho-c-Jun was performed on culture chamber slides using anti-phospho-c-Jun antibody, which specifically reacts with phosphorylated c-Jun. The cells were fixed in 4% paraformaldehyde for 10 min and then incubated for 1 hr in phosphate-buffered saline containing 1% bovine serum albumin and 0.2% Triton X-100, followed by incubation for 1 hr with a 1/150 dilution of anti-human phospho-c-Jun (Ser73) antibody and for an additional 1 hr with fluorescein (Oregon Green 514)-conjugated goat anti-rabbit IgG (Molecular Probes, Eugene, OR). The slides were mounted with aqueous mounting medium (Gelmount; Fisher, Pittsburgh, PA). Cultured cells were examined on an Axiovert 135 M microscope (Zeiss) with a laser scanning confocal microscope image system (Bio-Rad MRC-600), using a krypton/argon mixed-gas laser, with a filter allowing an excitation wavelength of 488 nm. For controls in every experiment, cells in an adjacent well were processed without primary antibody or secondary antibody. In all experiments, the control specimens did not exhibit any immunostaining.

Results

Fig. 1A shows that the D_1 dopamine receptor agonists SKF38393 (100 μM) and DHX (10 μM) induced approximately 2-fold increases in MBP phosphorylation. However, quinpirole, a D_2 dopamine receptor agonist, did not stimulate p38 MAPK activity. Levels of tyrosine-phosphorylated p38 MAPK were concomitantly increased in cells treated with the D_1 dopamine receptor agonists (Fig. 1B). Stimulation of p38 was observed after 5 min of incubation with 100 μM SKF38393 and was sustained for at least 120 min (Fig. 1C). Dose-dependent activation (1 μM, 1.5 ± 0.2-fold stimulation; 10 μM, 1.9 ± 0.3-fold stimulation; 100 μM, 2.2 ± 0.4-fold stimulation; 500 μM, 2.0 ± 0.5-fold stimulation) of p38 MAPK by SKF38393 was observed (Fig. 1C). SKF38393 also stimulated JNK activity (1 μM, 1.9 ± 0.2-fold stimulation; 10 μM, 1.9 ± 0.3-fold stimulation; 100 μM, 3.1 ± 0.4-fold stimulation; 500 μM, 1.5 ± 0.3-fold stimulation), exhibiting a time course and maximal stimulation level similar to those noted for p38 MAPK (Fig. 2, A and B). DHX also stimulated JNK activity (5-fold stimulation at 10 μM) (Fig. 2C). In agreement with the kinase activity data, immunocytochemical staining using a specific antibody that reacts with phosphorylated c-Jun [anti-human phospho-c-Jun (Ser73)] also demonstrated enhanced reactivity in cells stimulated with 100 μM SKF38393 (Fig. 3). However, the level of phospho-c-Jun reached its maximum at 15 min, whereas JNK activity, measured in immunoprecipitates, was maximally stimulated at 30 min. This small difference might be the result of activation of dephosphorylation pathways, which might decrease the accumulation of phosphorylated c-Jun. In contrast to the stimulation of p38 MAPK and JNK, the D_1 dopamine agonists did not activate ERK (Fig. 2D). Furthermore, 10 μM SCH23390, a specific D_1 dopamine receptor antagonist, blocked SKF38393-mediated JNK activation (Fig. 4A). However, because SCH23390 itself elevated basal p38 MAPK activity, an inhibitory effect of SCH23390 on this kinase was not as well defined (Fig. 4A). This elevated basal p38 MAPK activity produced by the antagonist might suggest constitutively active D_1 receptors in this cell line.

It has long been established that D_1 dopamine receptor stimulation activates adenylyl cyclase and increases intracellular cAMP levels. To investigate the possible role of cAMP in mediating the activation of p38 MAPK and JNK by D_1 dopamine receptor stimulation, the membrane-diffusible, cyclic nucleotide analogue dibutyryl-cAMP was used. Treat-

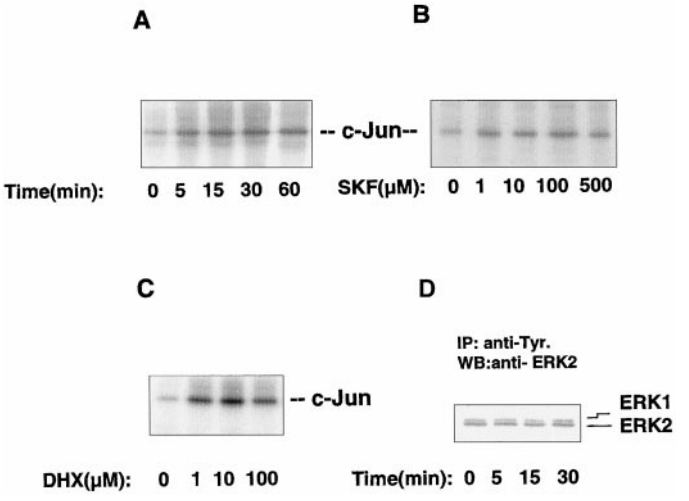

Fig. 2. A–C, Activation of JNK in SK-N-MC neuroblastoma cells by D_1 dopamine receptor agonists. Cells were treated with D_1 dopamine receptor agonists using the indicated times and doses. JNK activity was assayed as described in Experimental Procedures, using c-Jun-GST as the substrate. A, Time course for JNK activation by 100 μM SKF38393; B, dose-response data for SKF38393-mediated JNK activation (15-min incubation); C, activation of p38 JNK by 15-min incubation with DHX. D, Phosphorylation of ERK2, as assessed in 300 μg of cellular proteins. Samples were immunoprecipitated with antiphosphotyrosine (*anti-Tyr.*) antibody (4G10), precipitates were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred to nitrocellulose membranes, and phosphotyrosine protein-containing blots were probed with anti-ERK2 antibody. *IP*, immunoprecipitation; *WB*, Western blotting. Autoradiograms are representative of blots from three independent experiments.

ment of cells with 0.5 mM dibutyryl-cAMP mimicked SKF38393-mediated stimulation of p38 MAPK and JNK activities (Fig. 4, B and C). These effects were blocked by two structurally different PKA antagonists (i.e., H-89 and Rp-cAMPs). Furthermore, pretreatment with 1 μM H-89 (for 2 hr) or 10 μM Rp-cAMPs (for 20 min) completely inhibited the activation of p38 MAPK and JNK induced by 100 μM SKF38393.

The D_1 dopamine receptor is also known to couple to phospholipase C via G_q protein and to activate PKC (Rogue and Malviya, 1994; Wang *et al.*, 1995; Yu *et al.*, 1996). We therefore investigated the possible role of PKC in the D_1 agonist-induced activation of p38 and JNK. Incubation of cells with 100 μM SKF38393 for 15 min did not activate PKC, as determined by the absence of translocation of PKC from the cytosol to the membrane (cytosol, 8.3 $\pm$ 0.27 and 8.4 $\pm$ 0.09 pmol/min/μg of protein; membrane, 5.1 $\pm$ 0.13 and 5.3 $\pm$ 0.12 pmol/min/μg of protein, for control and 100 μM SKF38393, respectively). Moreover, the PKC inhibitor calphostin C (1 μM) did not affect SKF38393-induced activation of p38 MAPK and JNK (data not shown), indicating that PKC does not mediate the activation of these signaling pathways. Furthermore, because it has been reported that activation of MAPK by GPCRs requires tyrosine kinase activation (Blesen *et al.*, 1995; Wan *et al.*, 1996), we tested the effect of the tyrosine kinase inhibitor genistein on D_1 dopamine receptor-mediated p38 and JNK activation. As shown in Fig. 4D, the activities of p38 MAPK and JNK were not significantly inhibited by 100 μM genistein, suggesting that tyrosine kinase activation is unlikely to be a major modulator of D_1 dopamine receptor-mediated activation of p38 MAPK or JNK.

Discussion

Heterotrimeric G proteins mediate the transduction of signals from a variety of cell surface receptors to their effectors. Activation of ERK by GPCRs has been well documented (Koch *et al.*, 1994; Blesen *et al.*, 1995; Wan *et al.*, 1996; Yan *et al.*, 1997). The roles of GPCRs in the regulation of p38 and JNK signaling pathways were also recently reported (Rao and Runge, 1996; Jho *et al.*, 1997; Molnar *et al.*, 1997; Silva *et al.*, 1997; Voyno-Yasenetskaya *et al.*, 1997; Yamauchi *et al.*, 1997). The present investigation demonstrates for the first time that stimulation of the G_s-coupled D_1 dopamine receptor activates p38 MAPK and JNK in SK-N-MC neuroblastoma cells.

The signaling mechanisms for ERK activation by GPCRs involve Ras-dependent or -independent pathways and are determined by the type of receptor or by the associated G protein. G_i-coupled receptors mediate ERK activation

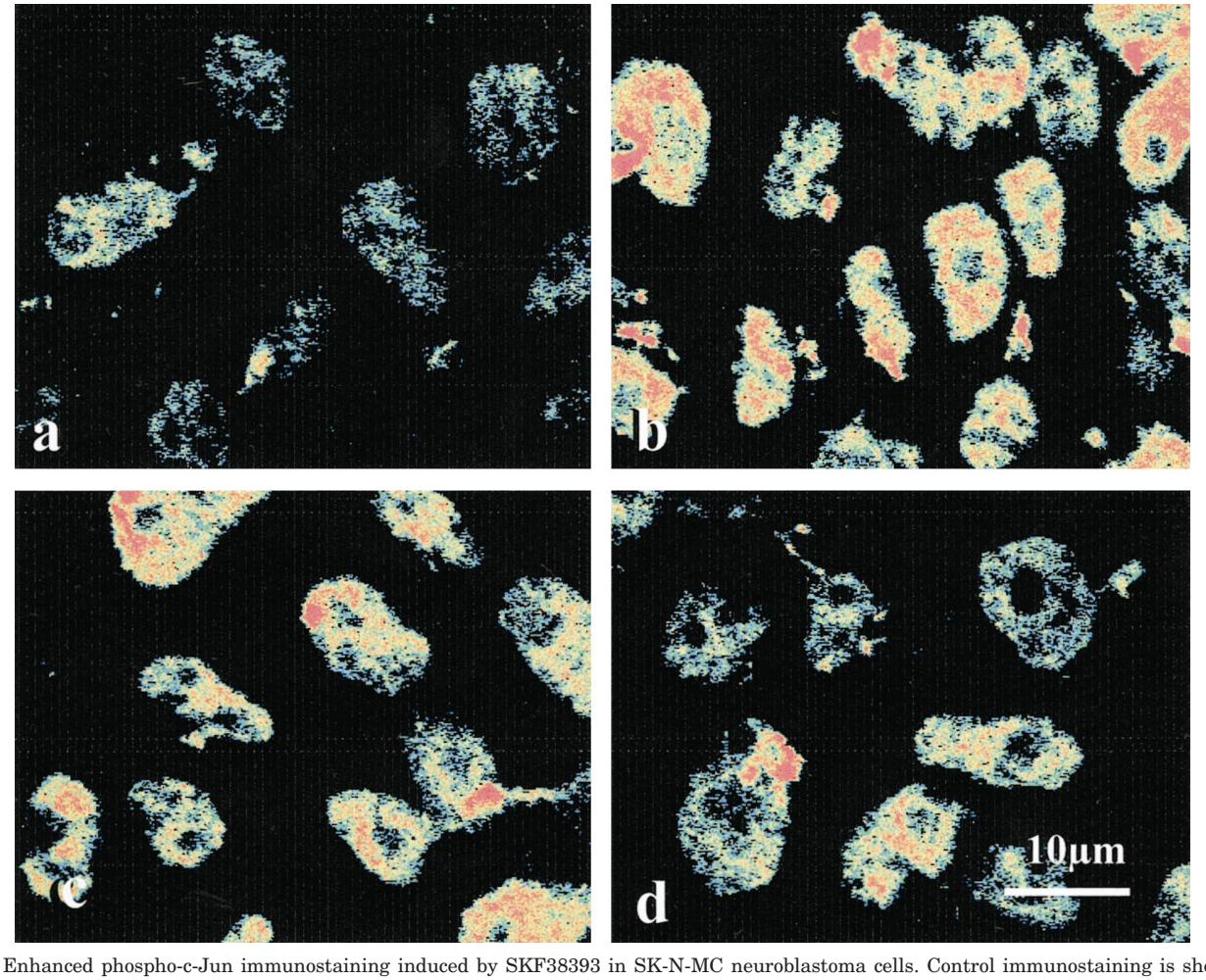

Fig. 3. Enhanced phospho-c-Jun immunostaining induced by SKF38393 in SK-N-MC neuroblastoma cells. Control immunostaining is shown (a). Cells treated with 100 μM SKF38393 for 10 min (b) or 15 min (c) exhibited increased immunostaining for phospho-c-Jun, but a decline toward control levels was noted after 30 min (d).

through a pathway that involves G protein $\beta\gamma$ subunits, Src tyrosine kinase, and Ras (Koch *et al.*, 1994; Crespo *et al.*, 1995; Wan *et al.*, 1996). G_q-mediated activation of ERK may require PKC and is independent of Ras activation (Crespo *et al.*, 1995). Although G_s was previously shown to activate ERK (Faure *et al.*, 1994; Post and Brown, 1996), in the present study we failed to demonstrate activation of ERK after D_1 dopamine receptor stimulation in SK-N-MC neuroblastoma cells. This may be the result of differences in cell types or different actions of specific receptors. Unlike that for ERK, the signaling pathways by which GPCRs activate p38 MAPK and JNK are poorly understood. Activation of p38 and JNK by m1/m2 muscarinic acetylcholine receptors was reported to be mediated by $G_{\beta\gamma}$ and/or $G_{q/11}$ (10); $G_{\alpha12}/G_{\alpha13}$ was also shown to mediate JNK activation via a Cdc42 pathway (Voyno-Yasenetskaya *et al.*, 1997), whereas G_s-coupled β-adrenergic receptors activate p38 MAPK mainly through $G_{\beta\gamma}$ (Crespo *et al.*, 1995). The present results demonstrate that D_1 dopamine receptor agonists activate p38 MAPK and JNK in SK-N-MC neuroblastoma cells, in a dose- and time-dependent fashion. PKA appears to mediate this stimulation, because activation of p38 MAPK and JNK in these cells is mimicked by a permeable cAMP analogue; moreover, inhibition of PKA markedly attenuates the activation of p38 MAPK and JNK that is evoked by D_1 dopamine receptor agonists. These observations contrast with the previous demonstration that cAMP inhibits thrombin-induced JNK stimulation in vascular smooth muscle cells (Rao and Runge, 1996). Different effects of PKA on the activity of the ERK signaling pathway were previously reported. For instance, PKA was shown to inhibit EGF-, platelet-derived growth factor-, and GPCR-mediated ERK activation in certain cell systems, by blocking Raf-1 activation, but was shown not to inhibit, or in some cases to stimulate, ERK activity in other cell systems (Wu *et al.*, 1993; Faure *et al.*, 1994; Cobbs and Goldsmith, 1995; Post and Brown, 1996). One possible explanation for these diverse actions of PKA might be related to differences in specific PKA isozymes involved in each of these pathways (Rao and Runge, 1996). At least two types of PKA isozymes (type 1 and type 2) have been identified, and these respond differently to stimulants (Taussig *et al.*, 1993; Rao and Runge, 1996).

The upstream molecules that mediate activation of p38 MAPK and JNK are not well defined, but it is clear that activation of the kinases relies on their phosphorylation at specific dual-phosphorylation motifs, namely the sequences Thr-Pro-Tyr for JNK and Thr-Glu-Tyr for p38 MAPK. The phosphorylation of these residues requires specific dual MKKs; available evidence indicates that MKK-3/6 is involved in the activation of p38 MAPK, whereas MKK-1/4 is the upstream activator of JNK (Paul *et al.*, 1997). The regulatory role of PKA in the signaling cascades is presently unknown. However, a recent study showed that activation of PKA resulted in phosphorylation of the β_2-adrenergic receptor and suppression of receptor/G_s coupling, with simultaneous enhancement of the coupling of β_2-adrenergic receptors to G_i; thus, the signaling pathway for this receptor was switched from G_s to G_i (Daaka *et al.*, 1997). This finding may suggest a new mechanism for the regulatory role of PKA in GPCR signal transduction. In addition, PKA might regulate the JNK or p38 pathways by activation of intermediate molecules. Indeed, it has been reported that the phosphorylated form of glia maturation factor, which is a product of PKA-mediated phosphorylation, markedly enhances the ability of glia maturation factor to activate p38 MAPK (Lim and Zaheer, 1996). Whether similar mechanisms operate in the D_1 dopamine receptor-mediated activation of p38 MAPK and JNK described in the present study remains to be determined.

The p38 MAPK and JNK signaling pathways have been suggested to play important roles in the regulation of cellular apoptosis. Recently, it was shown, in 293 cells and in primary striatal neurons, that dopamine elicits apoptosis by activating an oxidative stress-involved JNK signaling pathway (Luo *et al.*, 1998). It is interesting that dopamine receptor agonists that are used in the treatment of Parkinson's disease were

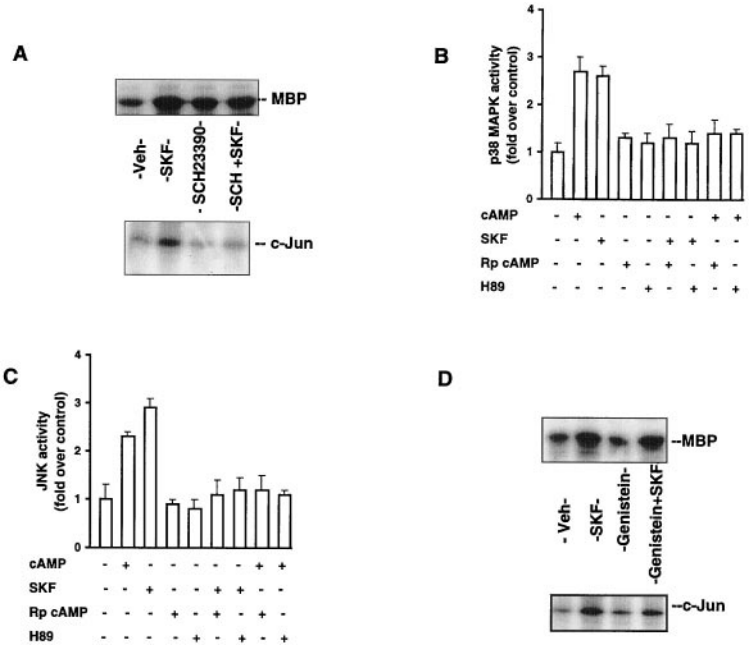

Fig. 4. Effects of analogues and antagonists on SKF38393-mediated stimulation of p38 MAPK and JNK. A and D, Cells that had been preincubated with 10 μM SCH23390 (*SCH*) (for 15 min) (A) or 100 μM genistein (for 2 hr) (D) were incubated with 100 μM SKF38393 (*SKF*) or vehicle (*Veh*), and p38 MAPK or JNK activities were determined after 15 min, using MBP or c-Jun, respectively, as the substrate. Autoradiograms representative of at least three separate experiments each are shown. B and C, Cells that had been preincubated with 1 μM H-89 (for 2 hr) or with 10 μM Rp-cAMPs (for 20 min) before a 15-min incubation with vehicle, 0.5 mM dibutyryl-cAMP (*cAMP*), or 100 μM SKF38393 were assayed for kinase activities by counting ^{32}P incorporated into the respective substrates [i.e., MBP for p38 MAPK (B) and c-Jun-GST for JNK (C)]. Data are the summaries of three independent experiments, expressed as mean ± standard error.

found to augment neuronal damage and promote neuronal apoptosis and may thus accelerate the progression of the disease (Gilmore *et al.*, 1995; Walkinshaw and Waters, 1995). The present demonstration that D_1 dopamine receptor stimulation activates the p38 MAPK/JNK pathways implicates these signaling pathways in the treatment-induced acceleration of parkinsonian pathological changes and/or the refractoriness to dopaminergic drug treatment.

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